Acta virol. **32** : 443—454, 1988

USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETERMINATION OF SEROLOGICAL RELATIONSHIP BETWEEN TWO COMOVIRUSES

J. GALLO, M. MUSIL

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

Received September 8, 1987

Summary. — ELISA was used to determine the degree of serological relationship between red clover mottle (RCMV) and broad bean stain (BBSV) viruses and the optimal conditions for the differentiation of the two viruses by ELISA were established. The titres of homologous and heterologous antibodies determined by ELISA were 100- to 200-fold higher as compared with the ring precipitin test. Antigen concentrations of 0.15 and 0.075 ng/ml, respectively, could be detected. The reactions in ELISA depended on the concentration of antigen, of IgG used for coating and of conjugated IgG. In anti-RCMV IgG, the ratio of group-specific to species-specific antibody as determined by ELISA was from 1 : 1 to 1 : 2, while in anti-BBSV IgG this ratio was from 1 : 4—1 : 8. The differences between homologous and heterologous reactions in ELISA suggested a similar ratio of antigenic determinants in the two viruses.

Key words: red clover mottle virus; broad bean stain virus; Comovirus group; serological relationship; ELISA

Introduction

Studies on serological relationships between members of individual plant virus groups and their strains remain in the centre of interest of plant virologists. The results of such investigations contribute to a general characterization of individual viruses and determine the possibilities of their reliable serological diagnosis.

Viruses of the Comovirus group are characterized by their serological relationship due to the occurrence in antisera of both species- and group-specific antibodies and to the presence of respective antigenic determinants (epitopes) on the surface of virions. On the virions of red clover mottle (RCMV) and broad bean stain (BBSV) comoviruses, a group of antigenic determinats species-specific for each virus is present along with a group of antigenic determinants shared by two or more viruses. Rabbit antisera against individual viruses contain groups of antibodies corresponding to

the respective antigenic determinants, which make possible a serological identification of the viruses and a determination of the degree of their serological (antigenic) relationship (Musil *et al.*, 1983). From this point of view we tested the suitability of the sensitive enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977), for studies on the serological rela-relationship between the two viruses. In comparative experiments we also attempted at establishing optimal conditions for a reliable differentiation between RCMV and BBSV by ELISA.

Materials and Methods

Viruses. In ELISA, purified suspensions of RCMV isolate TpM_{36} (Musil, 1973) and BBSV isolate VsM (Musil *et al.*, 1976) prepared as described (Musil, 1983) were used. Nucleoprotein concentrations in the virus suspensions were determined spectrophotometrically. In comparative experiments, we used virus suspensions of 0.01, 0.001, 0.00025 mg/ml, in some experiments also other concentrations.

Control wells contained purified pea enation mosaic virus or no antigen.

Immunoglobulin (IgG) fractions were prepared from hyperimmune rabbit antisera against RCMV isolate TpM_{36} and BBSV isolate VsM, the properties of which were described (Musil, 1973; Musil *et al.*, 1983; Musil and Gallo, 1985, 1986). The IgG fractions were isolated as proposed by Clark and Adams (1977), IgG having been separated on a DEAE 52 cellulose (SERVA) column. The IgG solution was adjusted to 1 mg/ml; a part of it was used for coating and the rest conjugated with alkaline phosphatase (type VII, SIGMA; 1 000 U per ml) as described by Clark and Adams (1977).

ELISA. The basic double antibody sandwich (DAS) type of ELISA was used throughout. It was carried out by the procedure of Clark and Adams (1977) on microtitration plates (KOH-I--NOOR, Dalečín, Czechoslovakia).

Wells of the plates were coated with anti-RCMV or anti-BBSV IgG; the initial solutions (1 mg/ml) were diluted 1 : 200, 1 : 1 000; 1 : 2 000; 1 : 4 000; 1 : 8 000; 1 : 16 000 and 1 : 32 000.

The conjugate solutions (1 mg/ml) were diluted 1 : 250, 1 : 500, 1 : 1 000, 1 : 5 000, 1 : 10 000 and 1 : 20 000 for antigen detection or up to 1 : 1 024 000 in antibody titrations.

Antigen, i.e. appropriately diluted RCMV or BBSV suspensions, was added in 200-µl volumes. After incubation for 4 hr at 37 °C with conjugated antibody, substrate [4-nitrophenyl phosphate bis(2-amino-2-ethyl-1,3-propanediol)-salt] was added in 210-µl volumes of diethanolamine buffer (pH 9.8) per well. The reaction was stopped after 30 min at 37 °C by the addition of 50 µl of 3 mol/l NaOH per well.

The results were evaluated spectrophotometrically by absorbancy measurements at 405 nm (Dynatech spectrophotometer) as described (Gallo and Valenta, 1985).

I. Reactions of RCMV and BBSV antigens with their homologous IgG

a) Dependence of the results on the concentration of antigen used

Samples of gradually diluted RCMV and BBSV antigens captured by homologous antibody adsorbed on the wells (IgG diluted 1 : 200 was used for coating) gave positive reactions with homologous labelled antibody (conjugate diluted 1 : 1 000) up to dilutions corresponding to nucleoprotein concentrations of 0.15 and 0.075 ng/ml, respectively. In general, the values obtained with gradually diluted antigens decreased proportionally to the degree of dilution (Fig. 1). More concentrated samples of both antigens gave reactions of similar intensity, while more diluted samples yielded

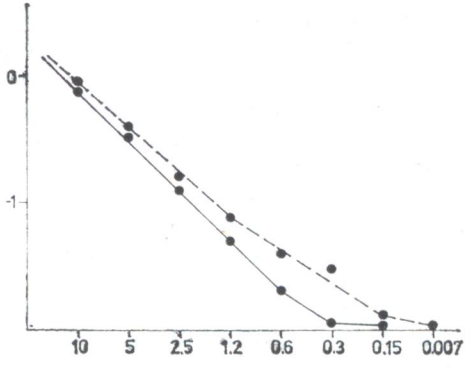

Fig. 1.
ELISA of gradual RCMV and BBSV antigen dilutions with homologous IgG Coat: IgG diluted 1 : 200; conjugate diluted 1 : 1 000.
Abscissa: antigen concentration (ng/ml); ordinate: absorbance at 405 nm ($\log_{10}$ values).
●– – –● RCMV, ●——● BBSV

values which for BBSV were lower than for RCMV. In comparative experiments we therefore used both antigens in concentrations of 100, 10, 5 and 2.5 ng/ml.

b) Dependence of the results on the concentration of conjugated IgG

In determining the titres of labelled antibody in the conjugates, we used two concentrations of homologous antigen (100 and 10 ng/ml) bound to homologous antibody used for coating (IgG diluted 1 : 1 000). In anti-RCMV conjugate, the antibody titre reached 1 : 512 000 with both antigen concentratios used. In anti-BBSV conjugate, this value for homologous antibody was only obtained with the more concentrated antigen; the lower antigen concentration yielded an antibody titre of 1 : 256 000. In general, the absorbance values in reactions of gradual IgG dilutions with 10 ng/ml of homologous antigen showed a linear dependence. More concentrated samples of BBSV and RCMV antigens reacted with conjugate dilutions up

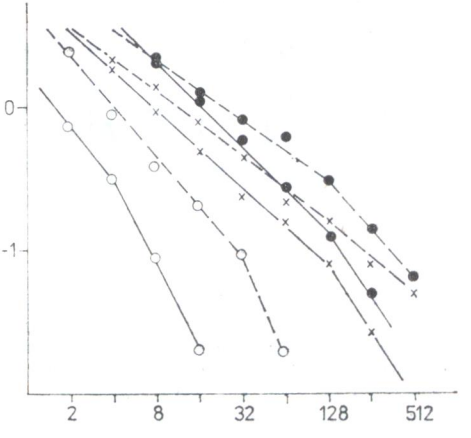

Fig. 2.
ELISA of gradual dilutions of anti-RCMV and anti-BBSV conjugates with homologous and heterologous antigens captured by homologous IgG coat
Abscissa: conjugate dilution $\times 10^{-3}$; ordinate: absorbance at 405 nm ($\log_{10}$ values).
RCMV–RCMV–RCMV (●– – –●) Ag 100 ng/ml
RCMV–RCMV–RCMV (●——●) Ag 10 ng/ml
BBSV–BBSV–BBSV (×– – –×) Ag 100 ng/ml
BBSV–BBSV–BBSV (×——×) Ag 10 ng/ml
BBSV–BBSV–RCMV (○– – –○) Ag 100 ng/ml
RCMV–RCMV–BBSV (○——○) Ag 100 ng/ml

to 1 : 32 000 (anti-BBSV IgG) and 1 : 64 000 (anti-RCMV IgG) by proportionally higher absorbance values as compared with more diluted antigens. This difference was manifested by a moderate change in the slope of the curves (Fig. 2). Absorbance values obtained with samples of less concentrated antigens were proportionally lower, with the exception of the results with limiting conjugate dilutions — the minimal absorbance values obtained with both antigen concentrations were almost identical (RCMV).

The results of reactions of anti-RCMV and anti-BBSV conjugates, diluted from 1 : 4 000 to 1 : 16 000, with a given antigen concentration were mutually almost the same. But the absorbance values in reactions of higher conjugate dilutions with individual concentrations of homologous antigens differed. The results suggested that the dilution endpoint of specific antibody in anti-BBSV conjugate was reached sooner than in the anti-RCMV conjugate (see Fig. 2).

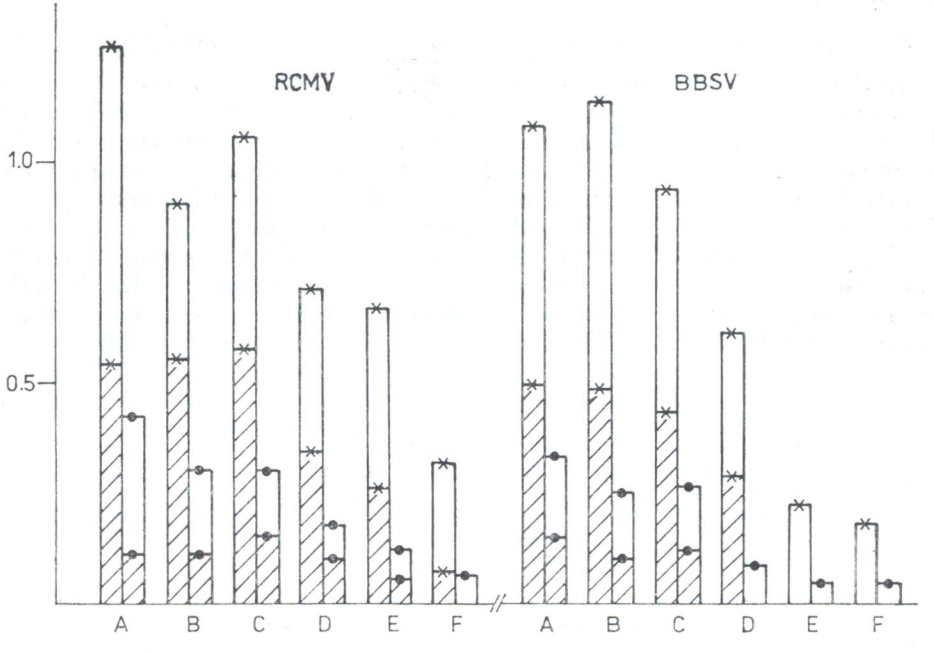

Fig. 3.
ELISA of anti-RCMV and anti-BBSV conjugates with homologous antigen captured by homologous IgG coats
Empty column: conjugate diluted 1 : 1 000; shaded column: conjugate diluted 1 : 5 000
Antigen concentration: 10 mg/ml (×), 2.5 ng/ml(●)
Abscissa: IgG used for coating diluted: A — 1 : 1 000, B — 1 : 2 000, C — 1 : 4 000, D — 1 : 8 000, E — 1 : 16 000, F — 1 : 32 000.
Ordinate: absorbance at 405 nm.

Table 1. Reactions in ELISA of anti-RCMV and anti-BBSV conjugates (diluted 1 : 5000) with homologous antigens

DAS ELISA Coat— Ag —conj.	Ag ng/ml	Absorbance at 405 nm					
		IgG used for coating diluted					
		1 : 1000	1 : 2000	1 : 4000	1 : 8000	1 : 16000	1 : 32000
RCMV—RCMV—RCMV	100	> 2.00	> 2.00	> 2.0	1.58	1.18	0.87
	10	0.53	0.54	0.57	0.34	0.26	0.07
	5	0 26	0.28	0.30	0.18	0.09	0 05
	2.5	0.11	0.13	0.15	0.10	0.05	0.04
BBSV—BBSV—BBSV	100	1.73	1.91	1.98	1.50	1.07	0.43
	10	0.49	0.44	0.43	0.28	0.22	0.17
	5	0.19	0.22	0.21	0.16	0.11	0.07
	2.5	0 05	0.12	0.12	0.08	0.04	0.04

c) Dependence of the results on the concentration of IgG used for coating

The effects of the concentration of the IgG used for coating were studied in reactions of anti-RCMV and anti-BBSV conjugates diluted from 1 : 1 000 to 1 : 20 000 with homologous antigens in concentrations of 100, 10, 5 and 2.5 ng/ml captured by antibody adsorbed on the wells. The IgG solutions used for coating were diluted 1 : 200 and then in 2-fold steps from 1 : 1 000 to 1 : 32 000.

In reactions of anti-RCMV and anti-BBSV conjugates (diluted, e.g., 1 : 1 000) with a certain concentration of homologous antigen, the absorbance

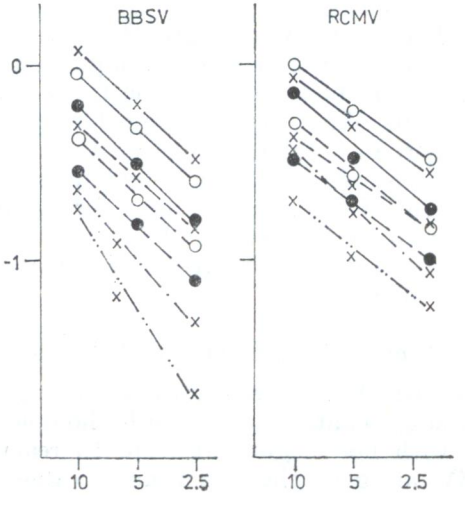

Fig. 4.
ELISA of various dilutions of anti-RCMV and anti-BBSV conjugates with homologous antigen bound to various concentrations of homologous IgG used for coating
IgG used for coating diluted 1 : 2 000 (×), 1 : 4 000 (○) and 1 : 8 000 (●).
Conjugate diluted 1 : 1 000 (————), 1 : 5 000 (— — — —), 1 : 10 000 (— . — . —) and 1 : 20 000(— .. — .. —)
Abscissa: antigen concentration (ng/ml); ordinate: absorbance at 405 nm ($\log_{10}$ values).

Table 2. Reactions in ELISA of anti-RCMV and anti-BBSV conjugates (diluted 1 : 1000) with homologous and heterologous antigens captured by IgG coat (diluted 1 : 2000)

Conjugate dilution	DAS ELISA Coat— Ag —conj.	Absorbance at 405 nm			
		Antigen concentration (ng/ml)			
		100	10	5	2.5
1 : 1000	RCMV—RCMV—RCMV	> 2.00	0.90	0.53	0.30
	BBSV —BBSV —BBSV	> 2.00	1.13	0.63	0.34
	RCMV—RCMV—BBSV	0.41	0.02	0.01	0.01
	BBSV —BBSV —RCMV	> 2.00	0.20	0.12	0.10
	BBSV —RCMV—RCMV	> 2.00	0.60	0.35	0.19
	RCMV—BBSV —BBSV	> 2.00	1.08	0.62	0.37
	RCMV—BBSV —RCMV	1.22	0.34	0 23	0 15
	BBSV —RCMV—BBSV	0 18	0.05	0.03	0.02
1 : 5000	RCMV—RCMV—RCMV	> 2.00	0.54	0.28	0.13
	BBSV —BBSV —BBSV	> 2.00	0.43	0.21	0.12
	RCMV—RCMV—BBSV	0.09	0.01	0.00	0.00
	BBSV —BBSV —RCMV	0.71	0.05	0.02	0.01
	BBSV —RCMV—RCMV	1.39	0.41	0.23	0.11
	RCMV—BBSV —BBSV	> 2.00	0.61	0.31	0.16
	RCMV—BBSV —RCMV	0.46	0.08	0.07	0.05
	BBSV —RCMV—BBSV	0.03	0.01	0.01	0.00

values were not or almost not related to the IgG dilution used for coating when the latter was in the range from 1 : 200 to 1 : 2 000. A dependence of the result of the reaction on the concentration of IgG used for coating was manifested only at higher dilutions (e.g., from 1 : 4 000 to 1 : 32 000) of IgG used for coating.

With anti-RCMV and anti-BBSV conjugates diluted 1 : 5 000 we obtained similar absorbance values with a given concentration of homologous antigen bound to antibody adsorbed on the wells from IgG dilutions from 1 : 1 000 to 1 : 4 000. When IgG dilutions higher than 1 : 4 000 were used for coating, the absorbance values of conjugates with any concentration of homologous antigen showed a 1.7- to 2-fold decrease, which approached the dilution coefficient of IgG solutions used for coating (Table 1, Fig. 3).

With all concentrations of IgG used for coating, the differences in results of reactions due to different concentrations of antigen and conjugate were preserved (Fig. 4).

II. Reactions of RCMV and BBSV with heterologous conjugates

Anti-BBSV and anti-RCMV conjugates diluted 1 : 1 000 reacted with heterologous antigen captured by homologous antibody coat less intensively then with homologous antigen. In reaction of anti-BBSV conjugate with RCMV antigen, the absorbance values reached only 2—10 % of those

obtained in reactions of similarly diluted antigen with homologous conjugate. Much higher absorbance values were obtained in reactions of anti-RCMV conjugate with BBSV antigen (30—60 % of values obtained with the same antigen samples and homologous conjugate).

Absorbance values obtained with anti-RCMV conjugate diluted 1 : 5 000 and BBSV antigen were from 4- to 6-fold lower than with conjugate diluted 1 : 1 000, but the differences related to the different antigen concentrations were preserved. Absorbance values in reactions of conjugates with heterologous antigens were substantially unaffected by the concentration of IgG used for coating when the latter was in the range from 1 : 1 000 to 1 : 8 000 In most repeated tests, anti-BBSV conjugate diluted 1 : 5 000 gave no more any positive reaction with 10 ng/ml or less of RCMV antigen. Only reaction of more concentrated RCMV antigen (e.g., 100 ng/ml) with such anti-BBSV conjugate gave measurable absorbance values. But such concentrated RCMV antigen samples yielded no positive reaction with higher dilutions (e.g., 1 : 10 000 or 1 : 12 000) of anti-BBSV conjugate. By contrast, the anti-RCMV conjugate diluted 1 : 10 000 reacted with BBSV antigen samples almost to the same absorbance values as did anti-BBSV conjugate diluted 1 : 1 000 with similarly concentrated RCMV antigen samples (Table 2).

Anti-RCMV and anti-BBSV conjugates diluted in 2-fold steps reacted with heterologous antigens captured by homologous IgG coat up to a dilution of 1 : 64 000 (anti-RCMV conjugate) or 1 : 16 000 (anti-BBSV conjugate). Within a conjugate dilution range from 1 : 2 000 to 1 : 16 000, the absorbance values in reactions of anti-RCMV conjugate with heterologous antigen were from 2- to 4-fold lower than with homologous antigen. Anti-BBSV conjugate diluted from 1 : 2 000 to 1 : 4 000 reacted with heterologous antigen samples by 2- to 4-fold lower absorbance values than with homologous antigen. In both conjugates there was a tendency to a steeper decrease in values of heterologous reactions with increasing conjugate dilutions as compared with homologous reactions (see Fig. 2). This more rapid decrease of absorbance values in heterologous reactions than in homologous reactions was due to a weaker reaction of group-specific antibody (present in lower concentration) with heterologous antigen as compared with the reaction of group-specific antibody together with species-specific antibody (present in higher concentration) with homologous antigen.

III. Binding of antigens to heterologous IgG coats

The absorbance values obtained in reactions of RCMV antigen captured by antibody of heterologous IgG coat with homologous conjugate reached 40—70 % of those in homologous reactions. With BBSV antigen, these values reached 40—100 % of the absorbance values obtained in homologous reactions (Table 3). In binding of antigens to antibodies of heterologous IgG coats and their detection by homologous conjugate, the differences in the representation in the IgG coats of the antibody group shared by both antigens was clearly manifested. The absorbance values in reactions of anti-BBSV conjugate (diluted from 1 : 1 000 to 1 : 20 000) with homologous antigen

Table 3. Reaction in ELISA of anti-RCMV and anti-BBSV conjugates (diluted 1 : 1000) with homologous and heterologous antigens

IgG dilution used for coating	DAS ELISA Coat— Ag —conj.	Absorbance at 405 nm			
		Antigen concentration (ng/ml)			
		100	10	5	2.5
1 : 2000	RCMV—RCMV—RCMV	> 2.00	0.90	0.53	0.30
1 : 4000		> 2.00	1.05	0.56	0.31
1 : 8000		> 2.00	0.76	0.36	0.18
1 : 2000	BBSV —BBSV —BBSV	> 2.00	1.13	0.63	0.34
1 : 4000		> 2.00	0.93	0.47	0.25
1 : 8000		> 2.00	0.61	0.32	0.17
1 : 2000	RCMV—RCMV—BBSV	0.41	0.02	0.01	0.01
1 : 4000		0.43	0.03	0.01	0.01
1 : 8000		0.39	0.03	0.01	0.01
1 : 2000	BBSV —BBSV —RCMV	> 2.00	0.20	0.12	0.10
1 : 4000		1.50	0.23	0.12	0.07
1 : 8000		1.02	0.22	0.12	0.06
1 : 2000	BBSV —RCMV—RCMV	> 2.00	0.60	0.35	0.19
1 : 4000		0.70	0.25	0.15	0.10
1 : 8000		0.27	0.10	0.06	0.05
1 : 2000	RCMV—BBSV —BBSV	> 2.00	1.08	0.62	0.37
1 : 4000		> 2.00	0.86	0.48	0.27
1 : 8000		0.78	0.33	0.19	0.11
1 : 2000	RCMV—BBSV —RCMV	1.22	0.34	0.23	0.15
1 : 4000		0.57	0.20	0.13	0.10
1 : 8000		0.19	0.09	0.05	0.04
1 : 2000	BBSV —RCMV—BBSV	0.18	0.05	0.03	0.02
1 : 4000		0.06	0.03	0.01	0.01
1 : 8000		0.03	0.02	0.01	0.01

bound to antibody of the anti-RCMV IgG coat (diluted 1 : 1 000—1 : 2 000) reached or even surpassed 100 % of the values in complete homologous reactions. This means that the same amount or more of BBSV antigen was bound by the respective group-specific antibody present in anti-RCMV IgG as compared with antibody present in homologous anti-BBSV IgG used for coating. By contrast, absorbance values in reactions of anti-RCMV conjugate with homologous antigen bound to group-specific antibody present in anti-BBSV IgG used for coating, reached only from 40 to 60 % of the homologous RCMV reaction. This means that the anti-BBSV IgG coat had a lower capacity to capture RCMV antigen, this capacity decreasing with dilution of the IgG used for coating more rapidly than in homologous reactions. For example both IgG coats used in a 1 : 8 000 dilution captured

significantly lower amounts of heterologous than of homologous antigen (see Table 3).

IV. Binding of antigen to antibody of heterologus IgG coat and its detection by heterologous conjugate

RCMV antigen captured by antibody of anti-BBSV IgG coat gave only a weak reaction with anti-BBSV conjugate. The absorbance values reached only 3—6 % of those obtained in homologous reactions (e.g. with a coat of IgG diluted 1 : 2 000 and conjugate diluted 1 : 1 000). At high dilutions of both coating IgG (e.g. 1 : 4 000—1 : 8 000) and conjugate (e.g. 1 : 5 000 to 1 : 20 000) the absorbance values were very low or no positive reactions could be detected (Table 3).

In reactions of anti-RCMV conjugate with BBSV antigen captured by anti-RCMV IgG coat, the absorbance values represented 40—60 % of those obtained in homologous reactions. With higher conjugate dilutions (e. g., 1 : 5 000—1 : 20 000) and the antigen concentrations used, the absorbance values were lower, proportionally to the degree of conjugate dilution. With higher dilutions of IgG used for coating (e.g., 1 : 4 000—1 : 8 000), the decrease of absorbance values obtained with captured heterologous antigen (especially RCMV) with heterologous conjugate was severalfold higher than in homologous reactions of corresponding antigen and conjugate concentrations (Table 3). The difference between the results of homologous and heterologous reactions was with more concentrated antigen usually smaller than with less concentrated antigen.

Discussion

The present results confirmed the great sensitivity of ELISA also for detection of comoviruses and for studies on their serological relationship. As distinct from other viruses, in which a high selectivity of ELISA in heterologous reactions was demonstrated (Koenig, 1978; Barbara *et al.*, 1978; Rochow and Carmichael, 1979; van Regenmortel, 1982; D'Arcy and Hewings, 1986; and others), with RCMV and BBSV we obtained in direct ELISA adequate homologous and heterologous reactions, corresponding to the level of the respective antibody groups in the IgG used for coating and in the labelled IgG and to the proportion of the respective antigenic determinants on the virion surface.

We confirmed by ELISA the presence in both anti-RCMV and anti-BBSV IgGs of two groups of antibody: group-specific antibodies are present in both IgG and are responsible for heterologous reactions; species-specific antibodies occur only in IgG against a certain virus and make possible a differentiation between virus species. Both antibody groups reached in ELISA substantially higher titres — 1 : 256 000—1 : 512 000 (species-specific antibody) and 1 : 16 000 and 1 : 64 000 (group-specific antibody in anti-BBSV and anti-RCMV IgG, respectively) — than were those found in the original antisera by the ring precipitin test (RCMV — 1 : 1 280/1 : 320, BBSV — 1 : 2 560/1 : 320; Musil *et al.*, 1983). The difference in titres of the two anti-

body groups in anti-RCMV and anti-BBSV IgG as detected by ELISA was from 8- to 16-fold, as compared to a 4- to 8-fold difference in titres of these antibodies in the original antisera found by the ring precipitin test. This was not only due to the difference between the two techniques in determining antibody titres, but probably also to a different proportion of virus-specific antibody in original antisera and in the IgG used in ELISA. While all precipitating virus-specific antibodies present in a given serum participate in the precipitation reaction, only virus-specific antibodies of the IgG class participate in ELISA and this with a different intensity as concerns capturing of antigen by the IgG coat and binding of labelled and unlabelled antibody to the given antigen. In view of the different titres of species-specific and group-specific antibodies in anti-BBSV and anti-RCMV IgG, the results of homologous and heterologous reactions depended greatly on the concentration of the conjugates used. To obtain similar absorbance values in homologous and heterologous reactions it was necessary to use a 5- to 20-fold more concentrated (less diluted) conjugate in the heterologous than in the homologous reactions.

The present experiments also confirmed the finding by other authors concerning the dependence of results of homologous and heterologous reactions on the antigen concentration used (Koenig 1978; Uyemoto, 1980; van Regenmortel, 1982; D'Arcy and Hewings, 1986; and others). In experiments of RCMV and BBSV we repeatedly found within a certain antigen concentration range in homologous reactions a direct relationship between the absorbance values and the antigen concentration used. In heterologous reactions, the absorbance values within the range of "optimal" antigen concentration also were proportional to the antigen concentration used, but the coefficient of difference between absorbance values was smaller then the actual difference between the respective antigen concentrations. This difference was due to both a lower concentration of group-specific antibodies in the labelled IgG used and a lesser representation of antigenic determinants of the virion surface. In heterologous reactions the possibility of binding of reactive antibody to corresponding antigen determinants is, therefore, much smaller than in homologous reactions, in which all available virus-specific antibodies and corresponding binding sites on the virions are participating.

The results of homologous and heterologous reactions were affected to a lesser degree by the concentration of IgG used for coating. We did not observe an inhibition of the homologous reaction by an excess of IgG coat (Koenig, 1978) but, like Koenig (1978) we also found it nesessary to use optimal lower concentrations of IgG for coating in establishing differences between homologous and heterologous reactions. The use of several IgG concentrations for coating in the first step, several antigen concentrations in the second step and at least two concentrations of conjugate in the third step increases the probability of an adequate evaluation of the results of homologous and heterologous reactions and thus of the serological relationship of the viruses under study.

A detailed evaluation of the effects of different concentrations of antigen, of the IgG used for coating and of the conjugate on the results of homologous and heterologous reactions between the two comoviruses made it possible to select optimal concentrations of the components for homologous and heterologous ELISA. Such tests made it possible to determine to what degree the final result was affected by the first step of the reaction, i.e.binding of antigen to the antibody coat, and the second step, i.e. binding of labelled antibody to antigen. The results of our comparative experiments confirmed that in homologous reactions both groups of virus-specific antibodies react with antigen, which leads to maximal absorbance values. By contrast, in heterologous reactions at the same concentrations of antigen and IgG as used in the homologous reaction, already in the second step only a part of virus-specific antibodies (group-specific antibodies) bind to antigen (antigenic determinants on the virions). A different ratio in the representation in the two viruses of antigenic determinants on the virion surface, corresponding to the respective antibody groups, affected the results of heterologous reactions especially in the third step, in which group-specific antigenic determinants, usually present on the virions in lesser amounts than species-specific antigenic determinants, can bind to group-specific antibodies, representing a minority of conjugated antibodies. In reactions in which antigens were bound by antibody from a heterologous IgG coat and detected by antibodies from a heterologous conjugate we therefore recorded the lowest absorbance values. This arrangement of ELISA also revealed a marked difference between anti-RCMV and anti-BBSV IgG as to the representation of group-specific antibodies. The difference between the two IgGs was manifested to a lesser degree also in unlabelled IgG used for coating. At a certain (1 : 1—1 : 2) ratio of group- and species-specific antibodies in the coat (e.g., anti-RCMV IgG), the latter can capture more heterologous antigen, reacting with homologous conjugate with the same or even greater intensity than in a homologous reaction. Higher absorbance values obtained in such partially heterologous reactions were probably due to a more suitable spatial configuration of the antigen bound to antibody from the heterologous coat which in the next step of the reaction made possible the binding of a greater amount of labelled antibody. A smaller proportion of group-specific antibodies in the IgG coat (e.g., 1 : 4—1 : 8 in anti-BBSV IgG) resulted in the binding of a smaller amount of heterologous than of homologous antigen. The difference in the representation of group--specific antibodies in the two conjugates was manifested by a more marked difference between the results of heterologous reactions of antigen with anti-BBSV conjugate as compared with anti-RCMV conjugate. Similar results of ELISA on other two viruses were reported by Koenig (1978), but she ascribed the differences to a lowered ability of antibody to bind to antigen due to labelling.

A comparison of the results of homologous and heterologous reactions (in combinations heterologous coat—antigen—homologous conjugate; or heterologous coat — antigen — heterologous conjugate; or homologous coat

antigen—heterologous conjugate) showed that in final ELISA readings the differences in concentrations or in the ratio of individual components interacting in homologous or heterologous reactions are added or multiplied. In anti-RCMV IgG we can anticipate a 1 : 1—1 : 2 ratio of group-specific antibodies to species-specific antibodies, while in anti-BBSV IgG this antibody ratio was 1 : 4—1 : 8. A similar ratio also applied to the representation of antigenic determinants in the respective viruses. An exact determination of the ratio of antigenic determinants in RCMV and BBSV would require the use of more appropriate techniques (e.g., indirect ELISA, ISEM, etc.). In general, however, the results described above demonstrated the possibility of using ELISA in studies on serological and antigenic relationship of two members of the Comovirus group. The results also suggested that on further optimization ELISA could be used in studies on serological relationships between members of other virus groups, in which the present ELISA techniques proved to be highly selective (Barbara *et al.*, 1978; Koenig, 1978; Rochow and Carmichael, 1979; van Regenmortel, 1982; Richter *et al.*, 1983; D'Arcy and Hewings, 1986).

References

Barbara, D. J., Clark, M. F., and Thresh, J. M. (1978): Rapid detection and serotyping of prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. *Ann. appl. Biol.* **90**, 395—399.

Bar-Joseph, M., and Salomon, R. (1980): Heterologous reactivity of tobacco mosaic virus strains in enzyme-linked immunosorbent assays. *J. gen. Virol.* **47**, 509—512.

Clark, M. F., and Adams, A. M. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. gen. Virol.* **34**, 475—483.

D'Arcy, C. J., and Hewings, A. D. (1986): Enzyme-linked immunosorbent assays for study of serological relationships and detection of three luteoviruses. *Plant Pathology* **35**, 288—293.

Gallo, J., and Valenta, V. (1985): Využitie modifikácií enzymoimunosorbentného stanovenia (ELISA) na detekciu vírusu výrastkovej mozaiky hrachu. *Sbor. ÚVTIZ — Ochr. rostl.* **21**, 1—6.

Koenig, R. (1978): ELISA in the study of homologous and heterologous reactions of plant viruses. *J. gen. Virol.* **40**, 309—318.

Musil, M. (1973): The effect of some buffers on the diffusion and precipitation of red clover mottle virus in the agar-gel double diffusion tests. *Biológia (Bratislava)* **28**, 741—751.

Musil, M., and Gallo, J. (1984): Serological and electrophoretic typing of red clover mottle virus isolates. *Acta virol.* **28**, 515—518.

Musil, M., and Gallo, J. (1986): Immunoelectrophoretic characteristics of four broad bean stain virus isolates. *Acta virol.* **30**, 332—336.

Musil, M., Lešková, O., and Kleja, Š. (1978): The occurrence of broad bean stain virus in the stand of vetch in Slowakia. (in Slovak.) *Sbor. ÚVTIZ — Ochr. Rostl.* **14**, 161—166.

Musil, M., Valenta, V., Kowalska, Cz., Wiatroszak, I., and Beczner, L. (1983): Serological properties of some comoviruses. *Biológia (Bratislava)* **39**, 231—236.

Richter, J., and Reichenbächer, D. (1983): Serologische Untersuchungen mit dem cucumber mosaic virus (Gurkenmosaik-Virus). IV. Verwandtschaftliche Beziehungen zu einem Stamm des peanut stunt virus. *Acta Phytopath. Acad. Sci. Hungaricae* **18**, 187—194.

Rochow, W. F., and Carmichael, L. E. (1979): Specifity among barley yellow dwarf viruses in enzyme immunosorbent assays. *Virology* **95**, 415—420.

Uyemoto, J. K. (1980): Detection of maize chlorotic mottle virus serotypes by enzyme-linked immunosorbent assay. *Phytopathology* **70**, 290—292.

Van Regenmortel, M. H. V. (1982): *Serology and Immunochemistry of Plant Viruses*. Academic Press, New York.